The relationship between turbidity and carotenoid-based coloration of centrarchid fishes in urban streams

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Agricultural and urbanization practices cause runoff of nutrients and sediments into aquatic systems, leading to elevated turbidity levels (i.e., amount of suspended particles in the water) and loss of aquatic biodiversity. Increased turbidity can alter visual environments through the differential scattering and absorption of light underwater. Therefore, in fishes that use visual cues to find mates, the effectiveness of visual signals can be compromised by turbidity and interfere with mating systems such that hybridization can occur. Carotenoid pigments responsible for red and yellow color patterns are energetically costly for fish to acquire from their diet. If signals are interrupted by increased turbidity, then obtaining and displaying carotenoid-based colors may not be profitable. Centrarchidae is a family of fish that uses carotenoid-based visual cues to help attract mates. Here, I tested for a relationship between increased turbidity and carotenoid-based coloration in several centrarchid fishes from degraded urban streams. I collected >200 centrarchids from low and high turbidity sites on each of the Olentangy (n=2) and Scioto Rivers (n=2) during spring, summer, and fall 2015. Total body red and yellow coloration was evaluated using a standard photographic technique for Bluegill (*Lepomis macrochirus*), Green Sunfish (*Lepomis cyanellus*) and their hybrids. There was a positive relationship between standard length and red-yellow coloration across all three groups. However, I found a strong negative relationship between turbidity and red-yellow color expression in Bluegill. Bluegill are used as an indicator of good water quality, and these results suggest their coloration is affected by turbid systems, which could lead to increased hybridization centrarchid species.

Introduction

Anthropogenic activity has greatly altered aquatic ecosystems. As an example, activities such as deforestation and agriculture can increase turbidity in water bodies with either sedimentary or nutrient inputs (van der Sluijs et al. 2011). Turbidity, the scattering and absorption of light due to suspended particulates in the water column, occurs naturally in waterways, and plays an important role in aquatic food webs and ecosystem functioning. However, elevated concentrations of particulates above natural levels are considered one of the most deleterious stressors on aquatic systems (Kemp et al. 2011). Increased particulate loads have the potential to lead to biodiversity loss (Seehausen et al. 1997, Kemp et al. 2011); freshwater biodiversity is an economically, scientifically, and educationally valuable natural resource, and has been positively correlated with ecosystem functioning. However, due to human activities in and near water, freshwater ecosystems are experiencing decreasing biodiversity at a much faster rate than terrestrial ecosystems (Ricciardi and Rasmussen 1999, Dudgeon et al. 2005). As biodiversity decreases, emphasis has been placed on understanding the mechanisms leading to species declines and how human-induced environmental change has affected these mechanisms (Mrosso et al. 2004).

Turbidity reduces the intensity of light and changes the spectral composition (i.e., color) of light in water (Collins and Hart 2014). Shifts in the intensity and wavelengths of light that penetrate the water column can alter the way fishes receive visual signals (van der Sluijs et al. 2011, Collins and Hart 2014). For example, nutrient and sedimentary pollution have both been found to narrow the spectrum of light that penetrates the water due to a loss of short wavelength light (blues and greens) higher in the water column and shift to long wavelength light (yellows and reds) deeper in the water column (Fig. 1, Levine and MacNichol 1982, Seehausen et al 1997, Kelley et al 2012). Under clear conditions, as light travels deeper into the water column, it shifts

towards short wavelength light ~450-500 nm whereas in the presence of algal and sedimentary turbidity light shifts towards long wavelength light more rapidly in the water column. Algal turbidity tends to change the color of transmitted light to ~550 nm-600 nm causing a green/yellow hue, and sedimentary turbidity tends to change the color of transmitted light to ~600-650 nm causing a red/brown hue (Fig. 1, Levine and MacNichol 1982).

In many sexually dimorphic species (i.e., species with phenotypic differentiation between females and males), females choose mates to increase their overall fitness. In the case of many fishes, females use visual cues from males like ornamentation, size, and courtship displays, to choose an appropriate mate in good condition (Barbosa and Magurran 2006). Kodric-Brown (1989) found that female guppies (*Poecilia reticulata*) preferentially mated with males that had more saturated red and yellow coloration, a trait found to be positively correlated with fitness, over males with more dull coloration.

While some color pattern components are fixed, many fishes have changeable coloration, which they can alter quickly for predator avoidance or communication with conspecifics, such as sexual selection cues (Kelley et al. 2012). The African cichlid, *Haplochromis burtoni*, and the Paradise Whiptail, *Pentapodus paradiseus,* can alter their coloration within 5 seconds and 0.25 seconds, respectively, by contracting pigments within their chormatophores (Muske and Fernald 1987, Mäthger et al. 2003). These quick color changes are considered physiological color changes and are caused by the motility of pigments or reflective structures within cells, occurring almost instantaneously. Alternatively, morphological color changes are a result of the accumulation of pigment concentrations in chromatophores and occur over longer time periods, on the order of days or weeks, and have a longer lasting effect on external coloration (reviewed in Leclercq et al. 2010).

Fishes of the family Centrarchidae (e.g., black basses and sunfishes) are distributed throughout eastern North America and are known for their diversity in nuptial coloration and intense courtship displays (Smith et al. 2015). Evidence that centrarchids use visual cues for sexual selection is found in a variety of species: for example, females show preference for more saturated, orange breast and cheek coloration in Bluegill (*Lepomis machrochirus*) (Cogliati et al. 2010a, Cogliati et al. 2010b), whereas longer opercular flaps are preferred in Longear Sunfish (*Lepomis megalotis*) (Goddard and Mathis 2000). Bluegill are sexually dichromatic during mating season and this color variation persists into the post-mating season (Warren 2009, Cogliati et al. 2010a). Higher color saturation has been found to correlate with male body condition (estimated using Fulton's condition factor, i.e., a measure of fish health that incorporates the weight and length of the fish) (Cogliati et al. 2010a). Females may therefore be choosing mates that display decidedly higher fitness by selecting for male ornamentation (Cogliati et al. 2010a).

In some species with color-based sexual selection, such as African cichlid fishes, female mate choice is associated with divergence in male coloration by way of directional selection resulting in reproductive isolation between color forms, thus promoting biodiversity (Maan et al. 2004). Cichlids can also exhibit disruptive selection by choosing to mate with two different color morphs at opposite ends of the color spectrum (e.g., blue and red) and avoid mating with intermediate color morphs, therefore selecting against hybrids (Stelkens et al. 2008). However, these selection patterns can be disrupted in altered light environments. Seehausen et al. (1997) reported that the massive loss of cichlid species in Lake Victoria was correlated with eutrophication of the lake (i.e., algal turbidity) and hybridization between closely related species. Therefore, it is important to understand how human-induced environmental changes, like

increased turbidity, affect signaling in aquatic ecosystems because these necessary signals can become harder to see through a cloudy or more turbid water column. The masking of signals can inhibit their reception, therefore affecting communication, reproduction, and survival of aquatic organisms (van der Sluijs et al. 2010).

Colorful sexual signals oftentimes include long wavelength, or yellow and red, components; a large amount of red, orange, and yellow coloration in the animal kingdom, including fishes, is caused by carotenoid pigments (Olson and Owens 1998, Leclerq et al. 2010, Svesson and Wong 2010, Pike et al. 2011, Sefc et al. 2014). Carotenoids are hydrocarbons that can only be synthesized by photosynthetic organisms, bacteria, and fungi, so animals rely on dietary acquisition for their carotenoid supply (Svesson and Wong 2010). Once acquired, in fish, these pigments can be found in the chromatophores of the skin (Gray et al. 2011). Carotenoid displays can be used for a plethora of reasons, including warning coloration, aggression, and species recognition (Svesson and Wong 2010). Long wavelength colors (yellow to red) are used for sexual selection in fishes and birds, with females preferring brighter and more saturated color signals (Blount 2004).

Carotenoids are not only responsible for coloration and sexual ornamentation, they also help with vision, act as antioxidants, aid in immune defense, and play a role in embryo development (Olson and Owens 1998, Blount 2004, Leclercq et al. 2010, Svesson and Wong 2010, Sefc et al. 2014). Carotenoid color expression is thought to be costly since fish cannot synthesize these colors but must acquire them from their diet. Thus, there is thought to be a tradeoff between coloration and immune functions, such that fish that display brighter colors are thought to be in better overall condition because they can afford to utilize carotenoids for signaling purposes instead of health functions (Olson and Owens 1998, Blount 2004, Sefc et al.

2014). Female cichlids have been found to use carotenoid coloration to determine fitness of prospective mates, for example preferring more-red males over less-red males (Maan et al. 2004). This study featured two different mate-choice experiments and a field study, concluding that the redness of males was the most influential variable when *Pundamilia nyererei* females chose mates. Their results also suggested that females could detect even small differences in carotenoid coloration.

In many fishes, and even amphibians (Secondi et al. 2007), as turbidity increases, the intensity of sexual ornamentation and courtship displays decreases and can cause relaxed or dishonest signaling (Seehausen et al. 1997, Järvenpää and Lindström 2004, Wong et al. 2007, Candolin et al. 2007). In normal conditions, male-male competition in fishes prevents poor-quality males from dishonestly signaling their condition to females Candolin 1999, Candolin 2000). For example, Wong et al. (2007) tested the effect of turbidity on conspecific competition in the Three-Spined Stickleback, *Gasterosteus aculeatus,* and found that there was no significant difference in the intensity of courtship effort between males in good vs. poor condition when exposed to algal turbidity, but there was a significant difference in clear waters. Relaxed and dishonest signals can block the mechanism (i.e., color-based mate choice) that results in reproductive isolation between species (Collins and Hart 2014). This can lead to hybridization and loss of species diversity because females have been found to mate indiscriminately with heterospecific males and conspecific males (Järvenpää and Lindström 2004, Witte et al. 2011).

In addition to the impact of masked signals for female mate choice, intraspecific competition between males can also be affected by increased turbidity. Male-male competition has been found to prevent dishonest signaling from low-quality suitors (Wong et al. 2007); however, when turbidity is high it may hinder sight of competitors. This might allow lower

quality males to signal dishonestly and hence reduce the evolutionary potential of sexual selection. Turbidity can also increase the time and energy spent on courtship and mate choice, which has relaxed the strength of selection on male nuptial coloration and courtship displays in some species (Candolin et al. 2007). These shifts can cause long-term effects on the evolution of male mating traits and female preferences (Candolin et al. 2007).

The issue of increased turbidity also poses problems for terrestrial and aerial predators of aquatic animals. For example, cormorants (*Phalacrocrax* spp.) tend to catch cichlids that are brighter in color and other piscivorous birds are more plentiful near clearer waters (Trewavas 1938). Overall, increased turbidity in aquatic systems could lead to lower biodiversity due to hybridization, (Seehausen et al. 1997) and potentially a breakdown of the aquatic to terrestrial food web, especially if aerial and terrestrial predators are unable to visualize their prey through the water.

Although the majority of literature points towards color signals becoming drabber in turbid waters, not all studies are in total consensus. Male Red Shiner (*Cypreinella lutrensis*) were found to exhibit redder fins in turbid conditions (Dugas and Franssen 2011). The authors' explanation for this included the non-territorial mating system of Red Shiner, interactions between the mechanism of color and the signaling environment, or reduced cost of color expression (e.g., because of reduced predation). Additionally, the level and consistency of increased turbidity may play a role in color expression. As with most organisms, so much variation exists both within and between fish species that we can expect some variation in the findings of these types of studies.

As part of a larger project that is evaluating aquatic to terrestrial linkages in turbid systems, my project is a baseline study to investigate if there is a relationship between turbidity

and coloration in the fish family Centrarchidae. The study of color has increasingly become more prevalent in the scientific literature; however, there is still much work that needs to be done in this field (Lim et al 2009). As aquatic ecosystems become more and more turbid, due to hardening of the watershed and a decrease of forest cover due to agriculture (Eyles et al. 2003, Känder and Seidler 2009), it is important to understand how human-induced changes will affect processes that occur in affected aquatic ecosystems in order to conserve species diversity.
I will specifically focus on two species of sunfish (Bluegill andGreen Sunfish) and hybrid sunfish to test the hypothesis that increased turbidity alters expression of carotenoid-based red and yellow pigments. Following the majority of the literature, I predict that as the level of turbidity increases, the saturation of color will decrease in centrarchids. This means that fish will display more saturated colors in clear waters and drab colors in murkier waters. The results for this study will help to inform future studies investigating expression of carotenoid-based pigments and aquatic to terrestrial linkages, as well as hybridization and loss of species diversity of centrarchids associated with turbidity in degraded urban streams.

Methods:

Fish were sampled at replicate sites on the Olentangy River (n = 2 sites) and Scioto River (n = 2 sites) during spring, summer, and autumn 2015 (Fig. 2). The Scioto River and Olentangy River are both part of the Scioto River watershed, which drains a total of 16,869 km^2. The Scioto River flows through central and southwestern Ohio, whereas the Olentangy River only flows through central Ohio. The mainstem of the Olentangy River joins the Scioto River in downtown Columbus. Both rivers drain a combination of agricultural and urban landscapes.

Several environmental variables were measured at each site. Three point-in-time turbidity samples were taken at each site every time fish were sampled, as were other environmental measurements, such as temperature, conductivity, and dissolved oxygen (Table 1). Turbidity samples were held in falcon tubes and taken immediately back to the lab for analysis. Turbidity was measured in Nephelometric Turbidity Units (NTU) with a Lamotte 2020E portable turbidity meter. The turbidity meter determines turbidity by shining a white light through a water sample and measuring the fraction of light scattered by suspended particles at a 90˚ angle to the light source.

We collected Bluegill and Green Sunfish, and their hybrids (Fig. 3) (n=153, 36, and 17, respectively, Table 1), using a bag seine and enlarged minnow traps. Upon capture, fish were housed in aerated stream water in order to minimize stress. Within 30 minutes, fish were placed in a clear, Plexiglas photo cuvette and photographed (following Maan et al. 2004) with a Canon G16 Powershot camera to evaluate overall body coloration. The cuvette featured a grey background and white standard so that photos maintain homogeneity.

After photos were taken, the weight (g) and standard length (cm) of each fish was measured. Fish were euthanized in a mixture of 2 ml clove oil solution (1:10 eugenol:ethanol) and 500 ml water (as approved in Dr. Suzanne Gray's IACUC Protocol #2014A00000055) and subsequently placed in Formalin for fixation. The sex of each fish was determined by dissection of the abdominal cavity, with males being identified by a set of testis and females being identified by a set of ovaries that featured eggs. Individuals that were too small to determine sex were classified as juveniles.

When defining color perception, three dimensions are commonly used (Munsell 1912; Endler 1990; Hofmann et al 2006). The three dimensions are hue (e.g., red vs. blue, etc.),

brightness (intensity of the spectral signal), and saturation (e.g., pink vs. red). Brightness refers to the amount of light from no light (black) to pure light (white) in any given hue. Saturation refers to the intensity of the hue from dull coloration to pure coloration. To evaluate overall body coloration of each fish, photographs were uploaded into Adobe Photoshop where the white standard in each photograph was used to balance brightness in order to hold it constant across all photos. The fins and eyes were cropped out and deleted to allow for analysis of the fish's body only (following Maan et al. 2004) (Fig. 4), because carotenoids are found in the chromatophores of the skin (Gray et al. 2011) and the fins and eyes of fishes have different reflectance values than the body of fishes. Once the bodies of the fish were isolated, the photographs were run through a data analysis script designed for the statistics program R (developed by Logan Smith). The script quantifies the number of total pixels, number of red pixels and number of yellow pixels in the photo, based on a set of threshold color criteria using the RGB color scale. Values used included red: hue= 0-26 plus 232-255 and yellow: hue= 27-45 (Maan et al. 2004) to hold hue constant across all photos. This allows the program to calculate the total percentage of yellow pixels (%yellow = total yellow pixels/total pixels) and the total percentage of red pixels (%red = total yellow pixels/total pixels) reflected from the body of the fish in the photograph. Since I was interested in assessing overall carotenoid coloration, I calculated the sum of %red+%yellow and used this metric to determine if the saturation of carotenoid-based colors are dependent on the independent variable, turbidity.

Analyses

To determine the differences in mean turbidity between sites I performed a one-way ANOVA with mean turbidity as the dependent variable and site as the independent variable for

the Olentangy River sites. I used a Welch's T-test to compare the mean turbidities on the Scioto River with mean turbidity as the dependent variable and site as the independent variable. Two different analyses were needed because the two Scioto sites had unequal variances and the Welch's T-Test is robust to unequal variances. This allowed for classification of sites as high turbidity or low turbidity sites. I performed a one-way ANOVA with amount of carotenoid coloration (%Red + %Yellow) as the dependent variable and species as the independent variable to test if species expressed different saturations of red and yellow coloration (pooled across seasons and sites). A Tukey HSD post-hoc test was used to determine pair-wise species differences in coloration. To test if turbidity influenced carotenoid coloration across species, I conducted a two-way ANOVA with turbidity (high or low) and species (Bluegill, Green Sunfish, and hybrid sunfish) as independent variables and carotenoid coloration as the dependent variable. Because of a significant interaction between species and turbidity, I also conducted three, separate one-way ANOVAs for each species with turbidity as the independent variable in order to determine which species' carotenoid coloration was influenced by turbidity. Additionally, I ran three separate linear regressions to test if standard length influenced the amount of carotenoid coloration expressed across species with carotenoid coloration as the dependent variable and log standard length as the independent variable.

Bluegill are an environmentally important species because they are an indicator of good habitat quality while Green Sunfish and hybrids are used to classify degraded systems (metrics from the Index of Biotic Integrity, Karr 1981). Since I had a sufficiently high sample size of Bluegill I was able to investigate their relationship with turbidity in more depth. To determine if season had an effect on Bluegill carotenoid coloration in high turbidity versus low turbidity sites, I first conducted a two-way ANOVA with turbidity and season (spring, summer, fall) as

independent factors and carotenoid coloration as the dependent variable. Because a significant interaction between turbidity and season existed, I also ran two separate one-way ANOVAs with season as the independent variable for both high and low turbidities. Additionally, I used Tukey HSD post-hoc tests to determine pair-wise differences in seasons.

Using a two-way ANOVA, I tested for differences in carotenoid coloration among sexes and turbidity levels. Although a significant interaction did not exist between sex and turbidity, there was still a significant difference in the amount of carotenoid coloration expressed between sexes. I used separate one-way ANOVAs to determine if there was a difference in the amount of carotenoid coloration displayed by male, female, and juvenile Bluegill in high turbidity sites and low turbidity sites, with sex as the independent variable and carotenoid coloration as the dependent variable. A Tukey HSD post-hoc test was used to determine pair-wise sex differences in coloration at high turbidity sites. I also ran two simple linear regressions for male and female Bluegill with carotenoid coloration as the dependent variable and log standard length as the independent variable to examine if standard length influenced both male and female carotenoid coloration. All analyses were performed using SPSS version 23.

Results

Both the Olentangy and Scioto Rivers had one consistently higher turbidity site and one consistently lower turbidity site (Table 1, Fig. 5). The Olentangy Wetlands and Scioto Inlet sites were high turbidity sites (both upstream of dams) and Olentangy Stadium and Scioto Dublin were low turbidity sites (both downstream from dams). Turbidity was found to change with season at all four sites, but some sites changed more than others (Table 2, Fig. 6). For example

the largest change in turbidity was at the Scioto Inlet site with an increase of 21.8 NTU from summer to autumn.

Standard length and carotenoid coloration (%Red + %Yellow) were log transformed to normalize data. Table 3 provides descriptive statistics for the amount of carotenoid coloration found in each species, sex, and at each site. Pooled across seasons and sites, Bluegill, Green Sunfish, and hybrid sunfish all displayed significantly different amounts of carotenoid coloration (Table 4, Fig 7). A significant interaction between species and turbidity influenced the amount of carotenoid coloration displayed in centrarchid fishes (Table 5), and further investigation showed that carotenoid coloration of Bluegill and hybrid sunfish was influenced by turbidity but carotenoid coloration of Green Sunfish was unaffected by turbidity (Table 6). Carotenoid coloration of both Bluegill and Green Sunfish were influenced by standard length, but hybrid sunfish were not (Table 7, Fig. 8).

Using a two-way ANOVA, I found a significant interaction between season and turbidity, indicating that carotenoid coloration of Bluegill was influenced by both factors (Table 8). Further analyses using separate one-way ANOVAs, indicated that Bluegill carotenoid coloration differed across all three seasons in low turbidity sites (Table 9, Fig. 9), decreasing in red and yellow saturation from the spring to the fall. However, Bluegill from turbid water sites did not differ in carotenoid coloration across time (Table 9). Individuals were classified as males, females, or juveniles based on sex determination criteria given above. A two-way ANOVA demonstrated that both sex and turbidity influence carotenoid coloration of Bluegill, but carotenoid coloration was not influenced by an interaction between sex and turbidity (Table 10). Tukey HSD post-hoc tests revealed that the only significant differences in the amount of carotenoid coloration were between males and juveniles and females and juveniles and not

between males in females in either turbidity level (Table 10, Fig. 10). Log standard length also influenced the amount of carotenoid coloration displayed in both male and female Bluegill, with males displaying slightly more, but not enough to be significant (Table 11, Fig. 11)

Discussion

My results suggest that turbidity plays a relatively large role in influencing coloration of Bluegill and hybrid sunfish, which supports my original hypothesis that turbidity affects the saturation of carotenoid based coloration in centrarchids. However, Green Sunfish coloration seems to be unaffected by turbidity, which could be due to the fact that they are a very tolerant species of sunfish (Karr 1981).

Additionally, the length of the fish was found to be positively correlated with the amount of red and yellow color expressed in Bluegill and Green Sunfish (see Fig. 8). This result is not unexpected as larger males may be more fit individuals with more energy for sexual display. Unfortunately, the sample size for hybrid sunfish is relatively low (n=17), and since the influence of standard length is approaching significance (p=0.077), a larger sample size may show that standard length affects the amount of carotenoid coloration in hybrid sunfishes also.

One interesting finding is that hybrid sunfish display significantly higher carotenoid coloration than Bluegill or Green Sunfish (Fig. 7). In past studies, more intense coloration has been an indicator of higher fitness in centrarchid fishes (Cogliati et al. 2010A). If this holds true for hybrid sunfish, then they may be at a competitive advantage over purebred sunfish. Crosses of the Pecos Pupfish (*Cyprinodon pecosensis*) with the Sheepshead Minnow (*C. variegatus*) have greater swimming endurance and faster growth rates than the Pecos Pupfish indicating that some

hybrids display vigor over parental species (Rosenfield et al. 2004). If sunfish hybrids are at a competitive advantage then sunfish biodiversity may be at risk.

Since Bluegill mate in the spring, we expect that they will express more saturated carotenoid coloration during spring. My results support this prediction in clear water environments; however, there was no difference in carotenoid coloration across seasons in turbid water environments. In this case, turbid conditions seem to cause Bluegill to relax their signaling. This relaxed signaling could lead to indiscriminate mating, and ultimately a decrease in overall fitness or increased numbers of hybrids. Alternatively, there could be movement of less colorful fish to more turbid areas during the spring where their chances of mating might increase due to dishonest signaling in a poor visual environment. These alternatives need to be investigated further using an experimental approach. For example, a mesocosm experiment which partitions the environment into natural light and monochromatic light microhabitats would allow for examining if more dull colored fish tend to mate in poor visual environments or not. It would be more ideal to actually subject the fish to turbid conditions, however I don't believe you could partition non-turbid and turbid areas while still allowing free movement of the test fish.

Other studies have found Bluegill to be sexually dimorphic in coloration (Warren 2009, Colgiati et al. 2010A); however, my results showed little difference in the carotenoid coloration of males and females in clear water or turbid water. This may not be surprising given that Bluegill males have three different mating strategies (parental, sneaker, and satellite males) (Gross 1991, Neff et al. 2003). Sneaker males mature early to attempt to intrude on parental nests during spawning in order to fertilize eggs; when they grow older and larger they take on a satellite male approach. Satellite males mimic female Bluegill and hover near parental nests in order to take advantage of spawning events and fertilize eggs (Gross 1991, Neff et al. 2003).

Parental males are the ones who guard and take care of the female's eggs, so these are the mates that females select. Sneaker and satellite males make up approximately 80% of male Bluegill populations (Neff et al. 2003), so simply using 'male' as variable may not be representative of parental male coloration differences from females Bluegill. Unfortunately, classifying males as parental, sneaker, or satellite was beyond the scope of this project. Future coloration studies focusing on Bluegill would benefit from focusing on parental males or at least distinguishing between the three types.

As a consequence of agricultural and urbanization practices, water bodies are becoming increasingly turbid in an ever-changing world. The results of this study may have many implications for Bluegill mating systems and aquatic to terrestrial food web structures. Increased turbidity could cause reverse speciation (e.g., a breakdown of reproductive isolating barriers) and result in a loss of biodiversity (Seehausen et al. 1997) due to relaxed signaling. This increase in turbidity can also affect aquatic to terrestrial food webs, as piscivorous birds have been found to prefer less turbid waters (Trewavas 1938). If aerial or terrestrial predators cannot see their aquatic prey, then catching aquatic prey becomes difficult, further impacting terrestrial biodiversity. In order to combat these emerging trends in turbidity and hybridization it is imperative that watershed and city managers attempt to divert runoff from entering straight into our waterbodies.

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Tables and Figures

Table 1. Mean (± 1 SE) environmental measurements at each site according to season.

Site/Season	Fish Sample Size (n)	Turbidity (NTU)	Temperature (°C)	Dissolved Oxygen (mg/L)	Conductivity (µS/cm)	Depth (cm)
a) Olentangy Wetlands	*44*					
Spring	24	18.7(1.0)	23.8(0.22)	5.9(1.34)	619(1.1)	40.8(8.0)
Summer	20	15.7(5.4)	24.1(0.03)	1.6(1.19)	622(1.5)	13.0(1.5)
Autumn	--	----	----	----	----	----
b) Olentangy Stadium	*66*					
Spring	41	9.8(0.8)	19.5(0.44)	3.2(1.00)	1005(271.8)	33.3(8.3)
Summer	18	5.0(0.5)	21.3(1.49)	3.6(1.04)	1044(167.4)	54.3(10.2)
Autumn	7	1.8(0.1)	11.7(0.28)	5.5(0.56)	602(136.3)	30.0(10.0)
c) Scioto Dublin	*40*					
Spring	17	3.7(0.2)	16.1(0.06)	8.9(0.06)	743(19.1)	58.3(4.4)
Summer	20	5.8(0.5)	22.0(0.78)	5.2(0.39)	575(11.5)	74.2(4.5)
Autumn	3	4.6(0.3)	15.9(0.18)	7.8(0.52)	594(6.6)	54.7(8.1)
d) Scioto Inlet	*56*					
Spring	18	5.7(0.3)	18.6(0.12)	8.1(0.13)	766(1.5)	83.3(8.3)
Summer	23	17.6(1.4)	22.7(0.00)	10.2(0.24)	579(0.3)	93.3(6.7)
Autumn	15	27.5(1.5)	15.1(0.03)	5.6(0.66)	702(1.9)	57.7(8.5)

Table 2. *a)* Two-sample T-Test comparing the mean turbidity of the two sites on the Olentangy River and *b)* Welch's T-Test comparing the mean turbidity of the two sites on the Scioto River. Two different analyses were needed because the two Scioto sites had unequal variances and the Welch's T-Test is robust to unequal variances.

	t	**df**	**Significance**
a) Olentangy River	2.798	6	0.031*
b) Scioto River	2.945	6	0.026*

Table 3. Descriptive Statistics for the amount of $\log_{10}$ transformed carotenoid coloration found in each species and sex studied, and at each site.

	Fish Sample Size (n)	Mean	Standard Error	Standard Deviation	Variance	Minimum	Maximum
a) Species							
Bluegill	153	-2.006	0.063	0.776	0.602	-5.740	-0.830
Green Sunfish	36	-2.344	0.137	0.820	0.672	-4.730	-1.000
Hybrid sunfish	17	-1.328	0.121	0.500	0.250	-2.450	-0.590
b) Sex							
Male	106	-1.783	0.065	0.668	0.447	-3.990	-0.590
Female	86	-2.157	0.086	0.802	0.643	-5.740	-0.740
Juvenile	14	-2.810	0.270	1.009	1.018	-5.500	-1.960
c) Site							
Olentangy Wetlands	44	-2.219	0.173	1.149	1.321	-5.740	-0.059
Olentagy Stadium	66	-1.853	0.095	0.772	0.596	-4.730	-0.830
Scioto Dublin	40	-1.972	0.270	0.101	0.407	-3.940	-0.930
Scioto Inlet	56	-2.054	0.072	0.538	0.289	-3.570	-0.970

Table 4. *a)* One-way ANOVA comparing the mean carotenoid coloration in all three species studied. *b)* Tukey HSD post-hoc test comparing all combinations of mean carotenoid coloration between species.

Source	Sum of Squares	df	Mean Square	F	Significance
a) ANOVA results					
Between Groups	11.934	2	5.967	10.172	0.000*
Within Groups	119.091	203	0.587		
b) Tukey HSD post-hoc test					
Bluegill vs. Green Sunfish					0.047*
Bluegill vs. hybrid sunfish					0.002*
Green Sunfish vs. hybrid sunfish					0.000*

Table 5. Full factorial two-way ANOVA comparing mean carotenoid coloration as a function of species and turbidity.

Source	Sum of Squares	df	Mean Square	F	Significance
Turbidity	0.271	1	0.271	0.501	0.480
Species	8.472	2	4.236	7.843	0.001*
Turbidity*Species	3.325	2	1.663	3.078	0.048*
Error	108.029	200	0.540		

Table 6. One-way ANOVAs comparing the means of carotenoid coloration displayed in clear vs. turbid water for each *a)* Bluegill, *b)* Green Sunfish, and *c)* hybrid sunfish.

Source	Sum of Squares	df	Mean Square	F	Significance
a) Bluegill					
Regression	8.623[a]	1	8.623	15.819	0.000*
Residual	82.877	151	0.549		
b) Green Sunfish					
Regression	1.287[b]	1	1.287	1.967	0.170
Residual	22.244	34	0.654		
c) hybrid sunfish					
Regression	1.092[c]	1	1.092	5.634	0.031*
Residuals	2.908	15	0.194		

a) R^2= 0.095 b) R^2= 0.055 c) R^2= 0.273

Table 7. Simple linear regression analysis of log standard length vs. carotenoid coloration for all three species of fish.

Source	Sum of Squares	df	Mean Square	F	Significance
a) Bluegill					
Regression	22.249[a]	1	22.249	48.470	0.000*
Residual	69.311	151	0.459		
b) Green Sunfish					
Regression	6.492[b]	1	6.492	12.956	0.001*
Residual	17.038	34	0.501		
c) hybrid sunfish					
Regression	0.774[c]	1	0.774	3.598	0.077
Residuals	3.227	15	0.215		

a) R^2= 0.243 b) R^2= 0.276 c) R^2= 0.193

Table 8. Full factorial two-way ANOVA comparing the mean carotenoid coloration of Bluegill as a function of season and turbidity.

Source	Sum of Squares	df	Mean Square	F	Significance
Season	27.25	2	5.031	5.031	0.007*
Turbidity	40.16	1	40.161	14.828	0.000*
Turbidity*Season	19.84	2	9.918	3.6619	0.028*
Error	398.15	147	2.708		

Table 9. Tukey HSD post hoc test determining pair-wise differences in carotenoid coloration between turbidities and seasons for Bluegill. This table features only significant differences.

Pair-wise comparison	Significance
Low Turbidity:Spring vs. Low Turbidity:Autumn	0.014*
Low Turbidity:Spring vs. High Turbidity:Spring	0.007*
Low Turbidity:Spring vs. High Turbidity:Summer	0.000*

Table 10. Full factorial two-way ANOVA comparing the mean carotenoid coloration of Bluegill as a function of sex and turbidity.

Source	Sum of Squares	df	Mean Square	F	Significance
Sex	46.25	2	23.125	8.938	0.000*
Turbidity	43.80	1	43.804	16.930	0.000*
Turbidity*Sex	14.99	2	7.494	2.897	0.058
Error	380.35	147	2.587		

Table 11. Simple linear regression analysis of log standard length vs. carotenoid coloration for male and female Bluegill.

Source	Sum of Squares	df	Mean Square	F	Significance
a) Male					
Regression	6.124[a]	1	6.124	15.372	0.000*
Residual	28.286	71	0.398		
b) Female					
Regression	4.987[b]	1	4.987	10.922	0.002*
Residual	30.591	67	0.457		

a) $R^2 = 0.178$ b) $R^2 = 0.140$

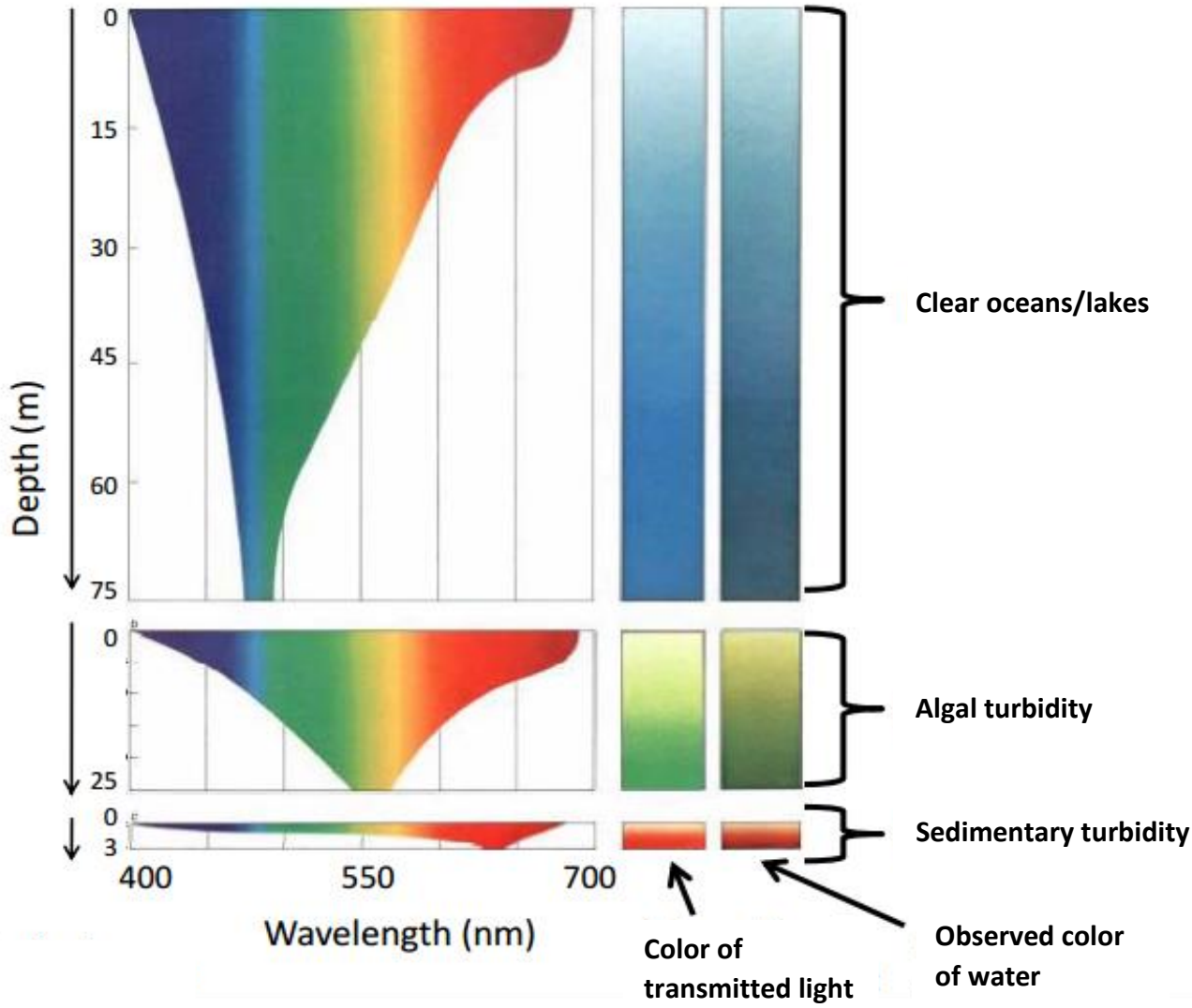

Figure 1. As light enters water, the clarity of the water determines how light will be transmitted through the water column. In clear waters the light becomes increasingly monochromatic and blue as its path length increases. In waters containing algal turbidity, light at all wavelengths is absorbed quicker and light becomes greener with path length. Waters containing sedimentary turbidity light at all wavelengths is absorbed rapidly and light becomes redder with path length (from Levine and MacNichol 1982 Fig. 2).

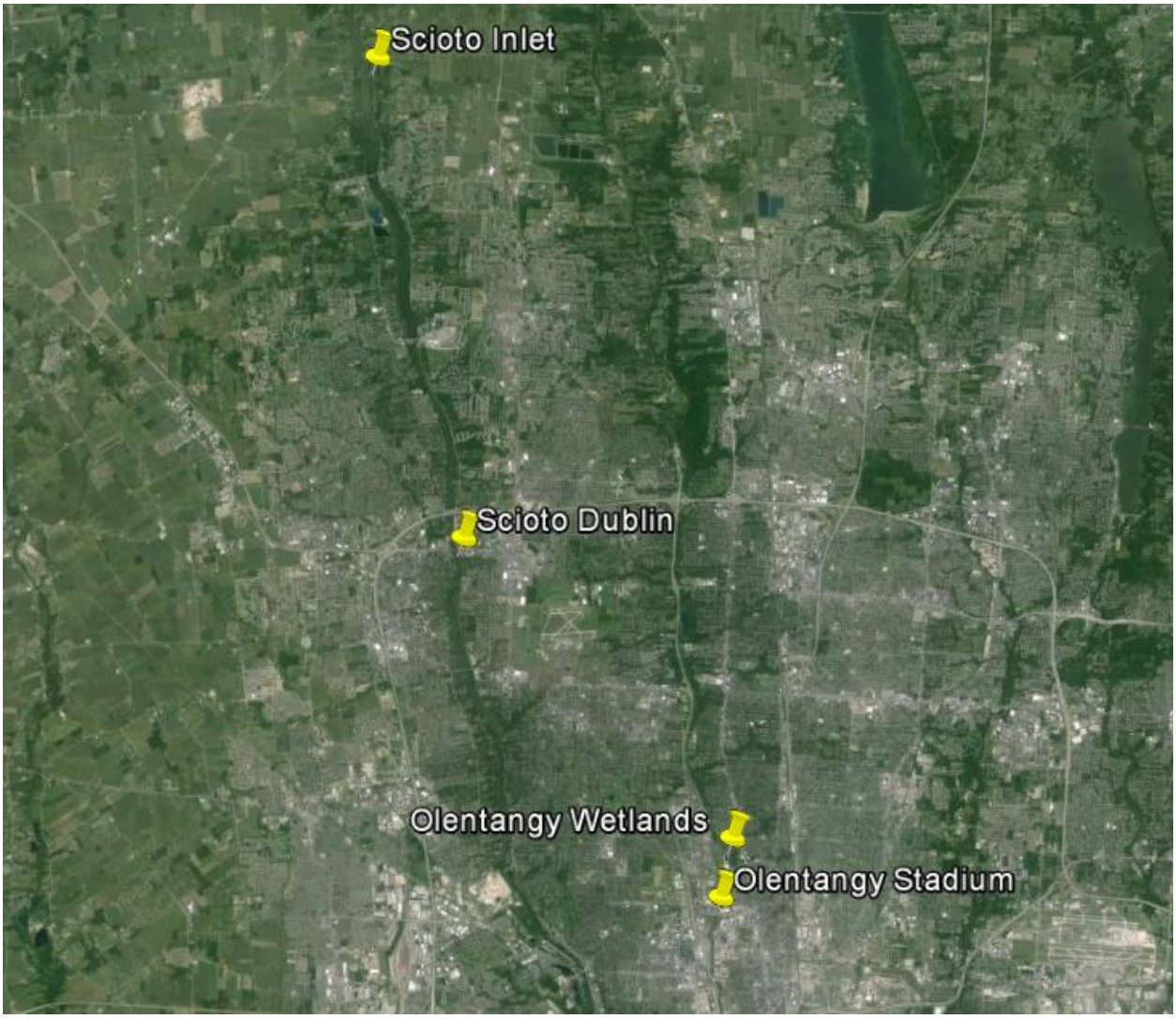

Figure 2. Fish were sampled at four sites on the Olentangy River (n = 2 sites) and Scioto River (n = 2 sites) during spring, summer, and autumn 2015 in the Columbus and Dublin areas. Upstream sites for each river are above a dam and downstream sites for each river are below a dam.

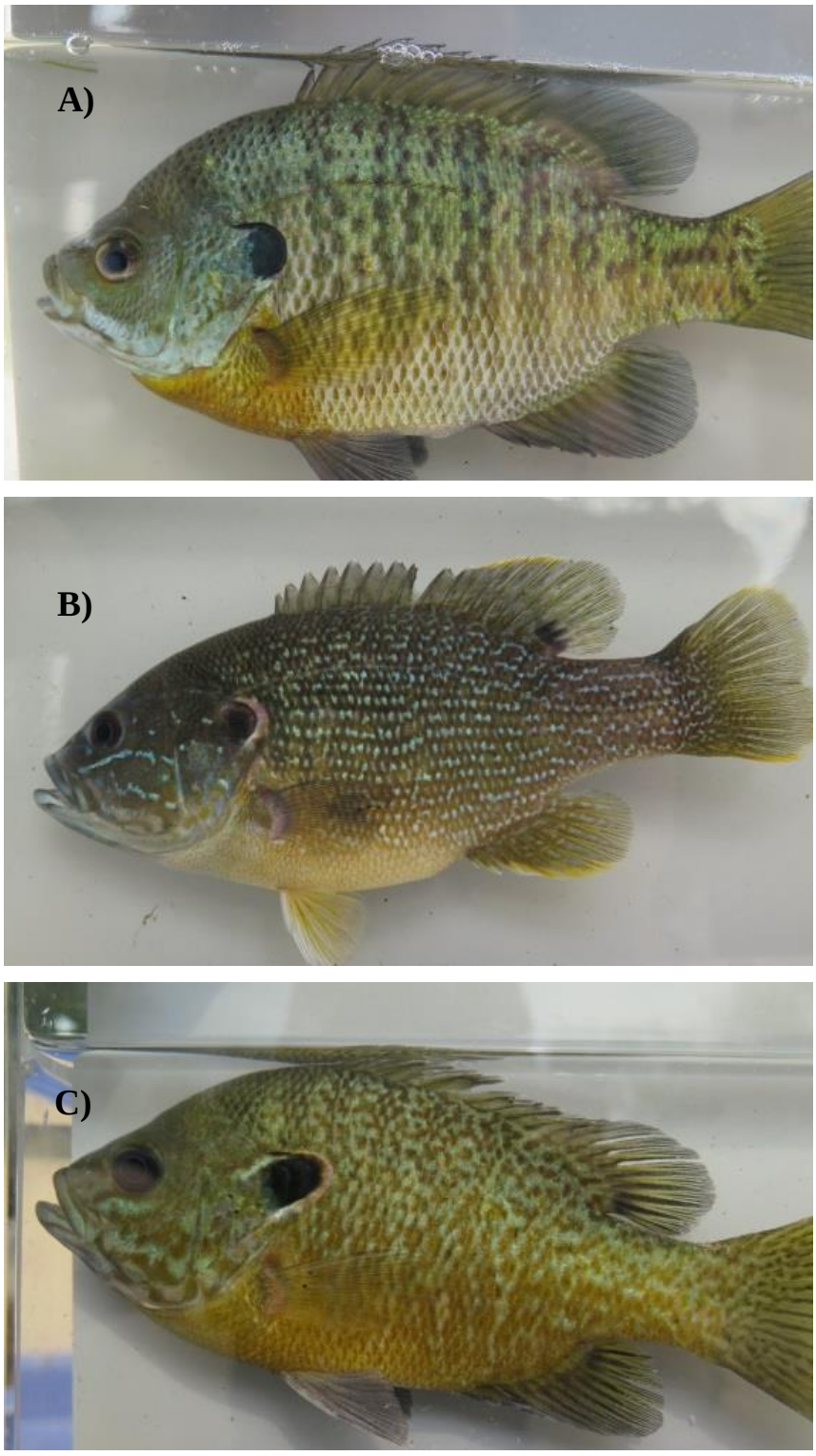

Figure 3. A) Bluegill (*Lepomis macrochirus*) **B)** Green Sunfish (*L. cyanellus*) and **C)** hybrid sunfish

A)

B)

Figure 4. The same male Bluegill in **A)** the initial photograph and **B)** after white balance and cropping in Adobe Photoshop.

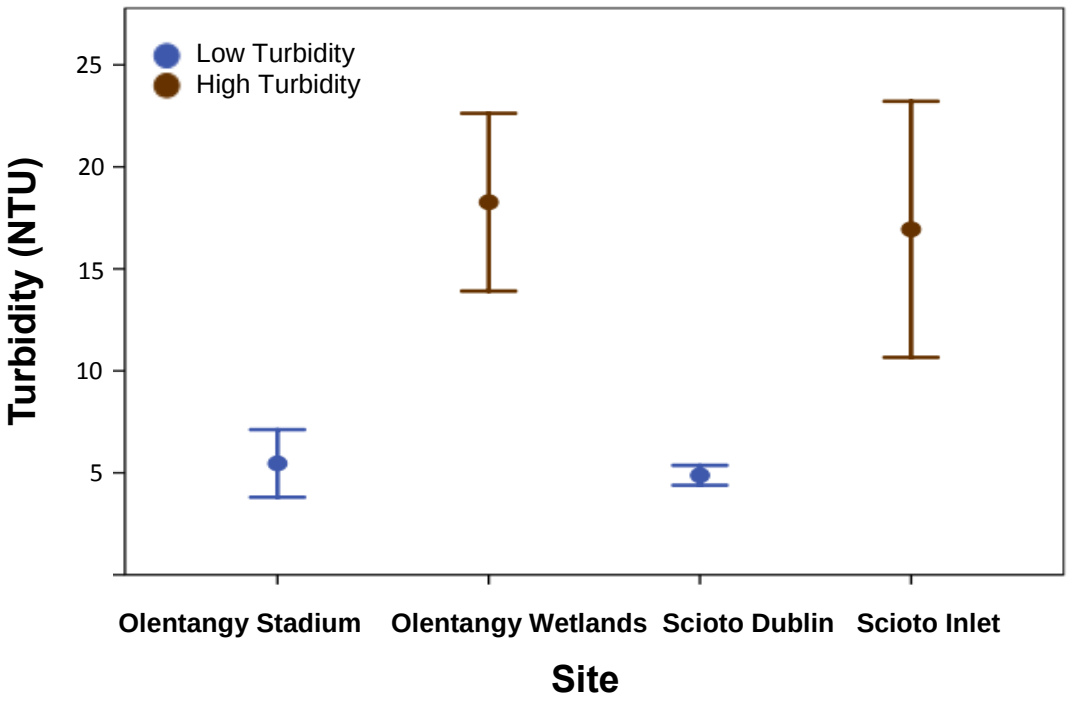

Figure 5. Mean turbidity (± 1 SE) across sites. Each river contains a significantly more turbid and less turbid site. Olentangy River: Two-Sample T-Test, t=2.798, p=0.032; Scioto River: Welch's T-Test, t=2.945, p=0.026.

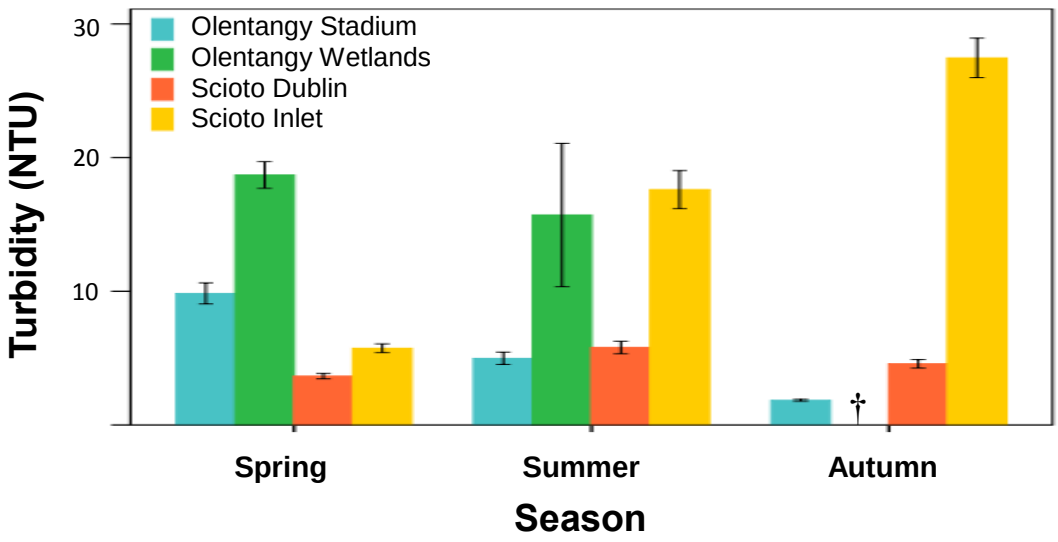

Figure 6. Mean turbidities (± 1 SE) of each site across seasons. †Due to unfortunate circumstances, Olentangy Wetlands was unable to be sampled in autumn 2015.

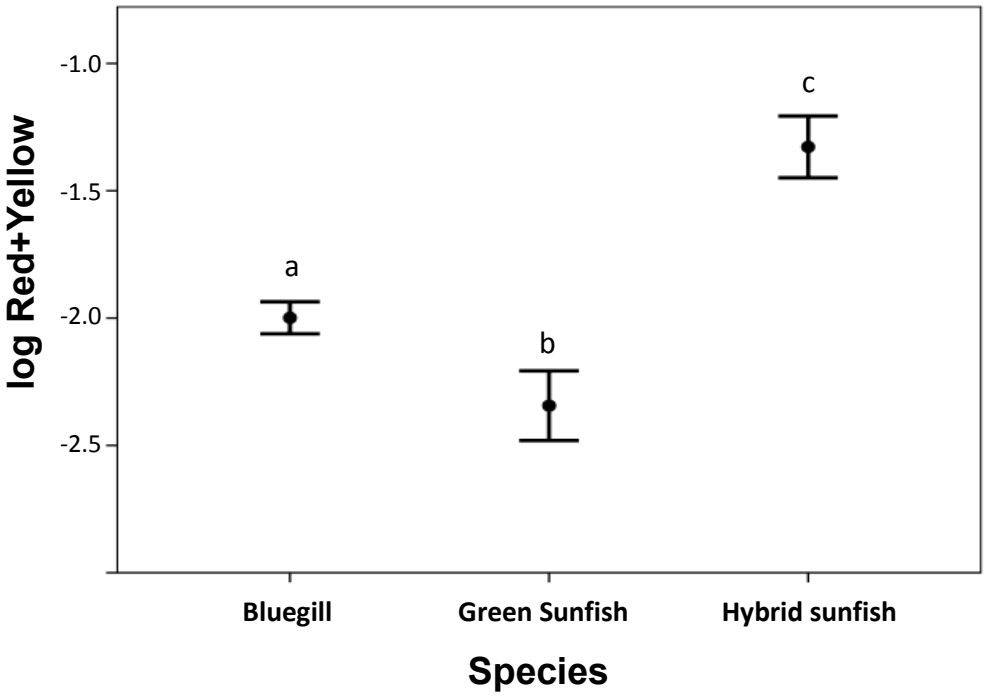

Figure 7. Mean carotenoid coloration (±1 SE) between species. One-way ANOVA, $F_{2,203}$= 10.17, $p<0.01$. The means of all three species are significantly differernt from each other, indicated by a, b, and c.

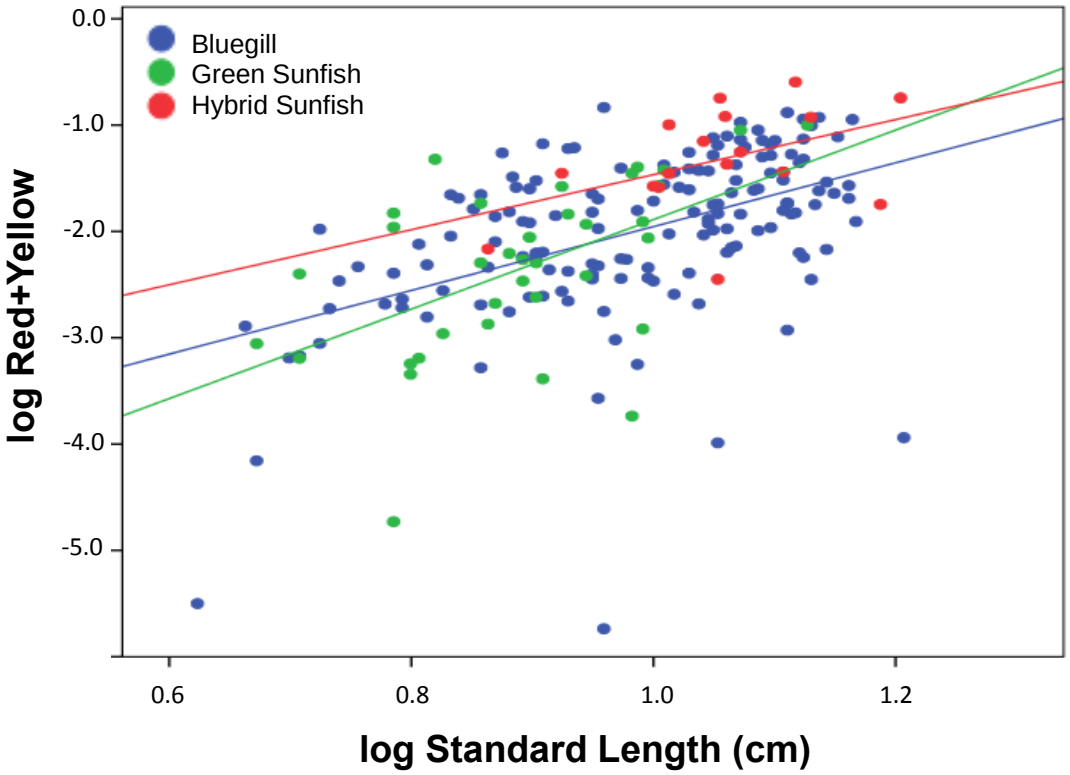

Figure 8. Regression analysis of log Red+Yellow as a function of log standard length by species. Bluegill: $F_{1,151}$=48.47, p<0.01, R^2=0.235, Green Sunfish: $F_{1,34}$=12.96, p<0.01, R^2=0.276, Hybrid: $F_{1,15}$=3.60, p=0.08, R^2=0.193.

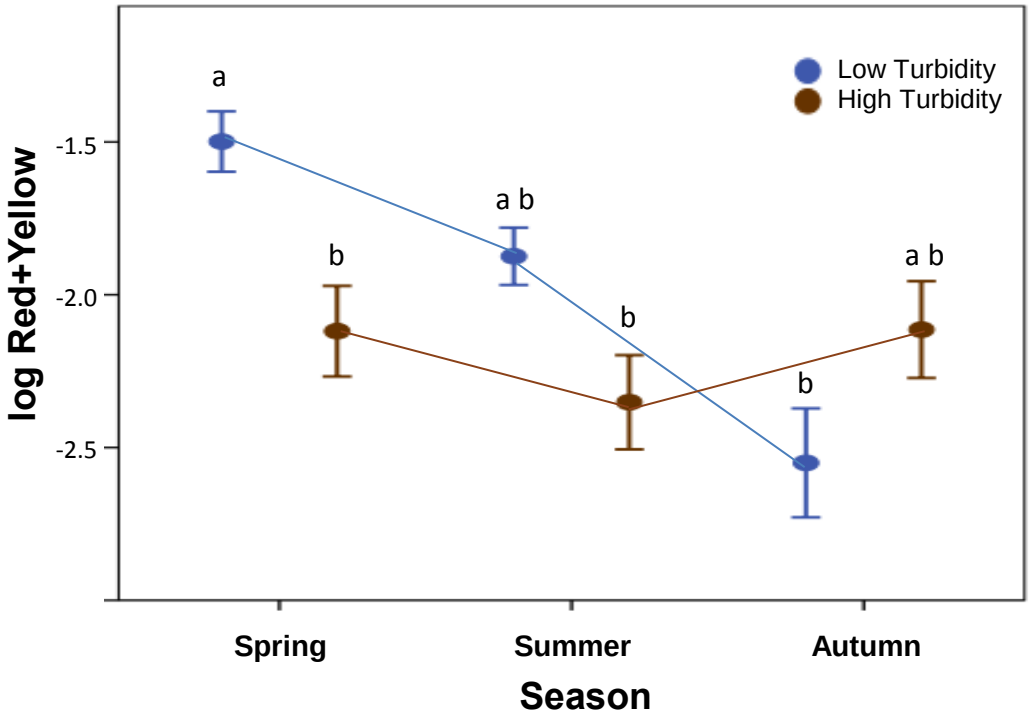

Figure 9. Mean carotenoid coloration (± 1 SE) of Bluegill across seasons, grouped by high turbidity (HT) and low turbidity (LT) sites. One-way ANOVA, LT: $F_{2,69}$=10.85, p<0.01; HT: $F_{2,78}$= 0.921, p=0.402.

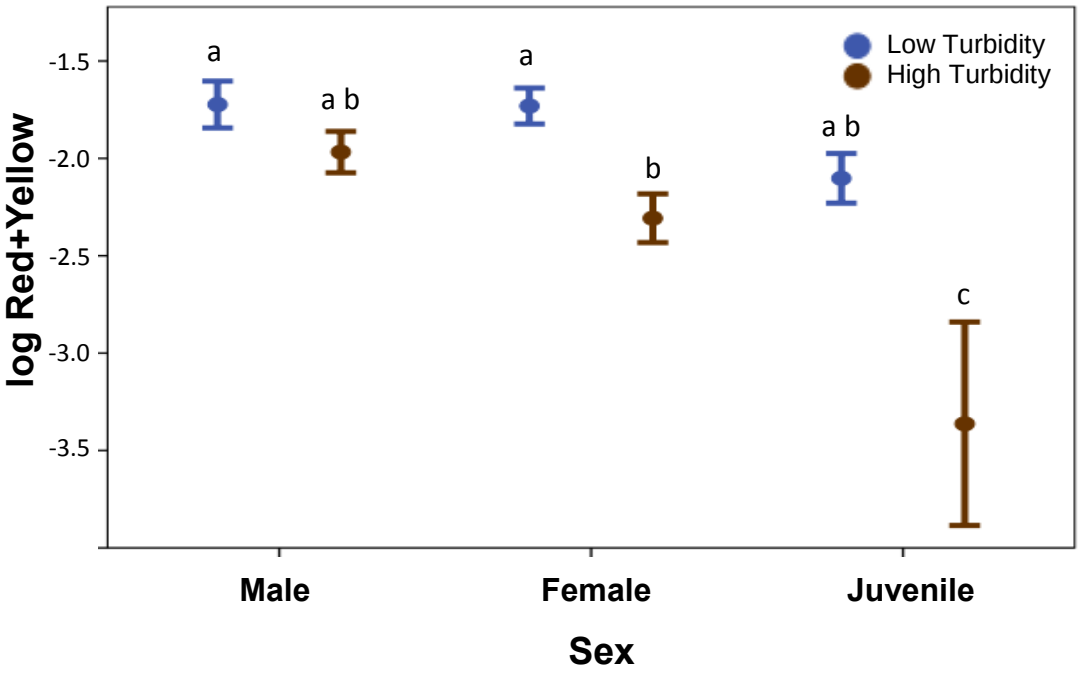

Figure 10. Mean carotenoid coloration (±1 SE) of Bluegill across sexes, grouped by high turbidity and low turbidity sites. One-way ANOVA, LT: $F_{2,69}$=0.856, p=0.429; HT: $F_{2,78}$=9.02, p<0.01. Letters indicate significant differences.

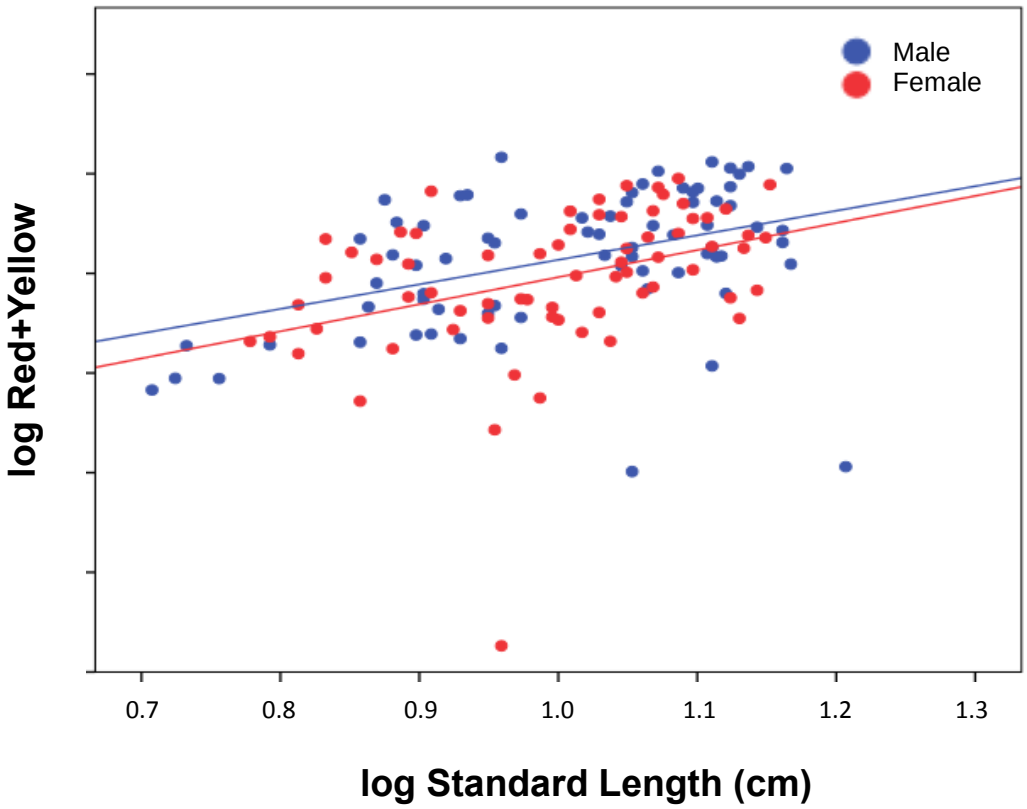

Figure 11. Simple linear regression of log Red+Yellow as a function of log standard length by sex in Bluegill. Male: $F_{1,71}$=1537, p<0.01, R^2=0.178, Female: $F_{1,67}$=10.92, p<0.01, R^2=0.140. Although this figure shows male Bluegill displaying more carotenoid coloration than female Bluegill, it is not a significant difference.